N-184: Phospholipid fatty acid analysis as phenotypic indicators of stress response in *Desulfovibrio vulgaris* and *Shewanella Oneidensis*

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Abstract

Metal reducing bacteria are often exposed to stressors in the environment which will affect their viability as well as their ability to reduce metal species of interest in bioremediation processes. Although genomic expression and proteomic analysis can provide great insight into microbial stress response, fundamentally it is interesting to understand how these stressors directly effect cell growth. Both Desulfovibrio vulgaris and *Shewanella oneidensis* were grown in batch culture and exposed a variety of stressors, including cold, heat, pH, salt, nitrate, and oxygen. The phospholipid fatty acids were extracted from the cultures at different time points to determine how the cell membrane responded to stress and to determine if specific fatty acid patterns can be used as an indicator of phenotypic response to stress analysis. It has been shown that during salt stress, Desulfovibrio vulgaris increases its amount of lipid per cell, and at the same time increases its proportion of saturated lipids. During oxygen stress of *D. vulgaris*, no growth occurs in the cells, but no significant death occurs and there is little or no change in the lipid patterns or total amount of lipids in the culture. For *Shewanella oneidensis*, many genes that are involved in production of saturated and/or branched-chain fatty acids are affected by both temperature and salinity. Our hypothesis is that these changes reflect the molecular mechanism by which the cells adapt their membrane fluidity to external conditions.

Methods--Growth

0.5M NaCl stress – D. vulgaris and *S. oneidensis*

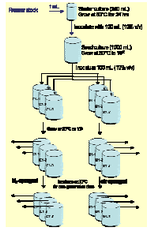
Triplicate separate freezer stock cultures *Shewanella oneidensis* MR1 (SO) from the same ATCC lot were grown in 3 x 100 ml LS4D medium at 1% inoculum. The cells were incubated aerobically at 200 rpm at 30°C. When cells reached mid log phase, cells were subcultured 10% into fresh LS4D into 6 x 1 liter flasks, one labeled as control and one as the stress. The final volume of liquid in all flasks was 200 ml.
When subcultured cells reached mid log, a 30 ml T0 sample was taken from all flasks. Shock stress was initiated by the addition of 16.7 ml of 5 M NaCl with a parallel addition of 16.7 ml of sterile DI water to the control bottles. 30 ml samples were taken at 1, 2.5, 5 and 10 hours after the addition of stress.
For DvH, the experimental protocol was similar but 2000 mL cultures were incubated anaerobically in bottles. Time points were taken 0, 0.5, 1, and 4 hours after the addition of oxygen.

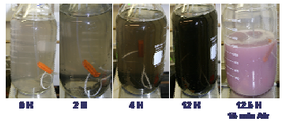
Cold stress. 8°C– *S. oneidensis*

Triplicate separate freezer stock cultures SO from the same ATCC lot were grown in 3 x 100 ml LS4D medium at 1% inoculum. The cells were incubated aerobically in a shaker incubator maintained at 30°C. When cells reached mid log phase, cells were sub cultured 10% into fresh LS4D into 6 x 1 liter flasks, one labeled as control and one as the stress. The final volume of liquid in all flasks would be 200ml.
When subcultured cells reached mid log, a 30 ml T0 sample was taken from all flasks. Shock stress was initiated by the transfer of the stress cultures through a heat exchanger to new sterile flasks. The heat exchanger and the receiving flasks were maintained at 8°C. The control samples remained on a shaker at 30°C. This method was able to drop the temperature in the entire SO culture to the desired temperature in less than 2 minutes. 30 ml samples were taken at 0, 45, 75, 135 minutes after the start of the cold stress. All samples were analyzed for phospholipids and OD (600 nm).

Oxygen stress—*D.vulgaris*

Triplicate separate freezer stock cultures *DvH* from the same ATCC lot were grown in 3 x 400 ml LS4D medium at 1% inoculum. The cells were incubated anaerobically at 30°C. When cells reached mid log phase, cells were subcultured 10% into fresh LS4D into 6 x 2 liter bottles equipped with sparging caps, three labeled as control and three as the stress. The final volume of liquid in all bottles was 2000 ml. The bottles were sealed, then removed from the anaerobic chamber and incubated for ~12 hrs in a 30°C water bath.
When subcultured cells reached mid log, a T0 sample was taken from all bottles by pushing out the culture with N_2 gas. Shock stress was initiated by sparging the bottles with N_2 gas containing O_2. Several experiments were run, ranging from 0.5 to 20.9% O_2. The control bottles were sparged with N_2 gas. Samples were taken 0, 0.5, 1, and 4 hours after the addition of oxygen.



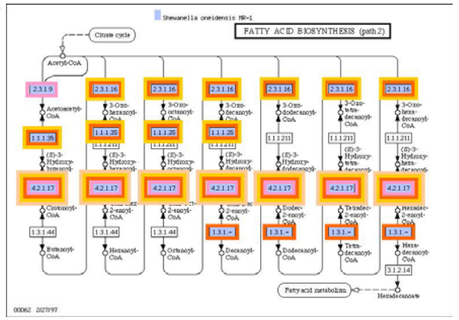


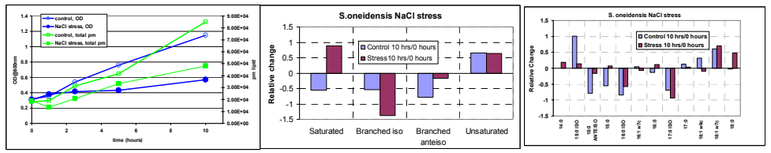
D. vulgaris cultures after exposure to 20.9% oxygen. Resazurin in the medium caused the culture to turn pink after 15 minutes of sparging with air. The cultures sparged with 0.1% did not turn pink.

Phospholipid response to NaCl stress

***Shewanella oneidensis* MR1 (SO)**

Based on a genome study of *S. oneidensis*, we created a diagram describing the Fatty Acid Biosynthesis pathways. Many of these steps are likely involved in production of saturated and/or branched-chain fatty acids. Genes under the influence of this regulator respond to changes in both temperature and salinity. Our hypothesis is that these changes reflect the molecular mechanism by which the cells adapt their membrane fluidity to external conditions that might affect it. Because these changes are correlated with salt/pH/temperature, the expectation is that, consistent with previous reports in the literature, these changes in FA are reflected in the phospholipid content of the membrane.

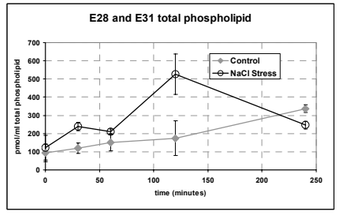


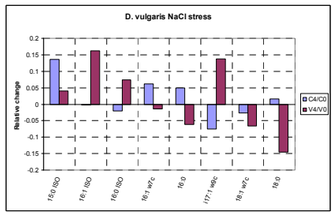


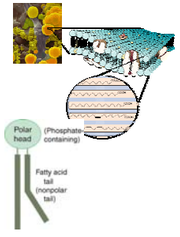
SO was grown in the presence of 0.5 M NaCl. The plot above shows growth of SO over time after the addition of stress, shown as both OD and lipid recovered. The addition of 0.5M NaCl decreased the rate of growth in the culture. Lipid composition was compared between samples taken at 0 hrs and 10 hrs. The composition of lipid changes in both the control and stressed cells during growth. However, there are distinct differences in cell membrane composition can be observed due to NaCl stress. Unlike DvH, the saturated lipids show and overall increase (esp 18:0) and the branched iso lipids show a decrease.

Desulfovibrio vulgaris

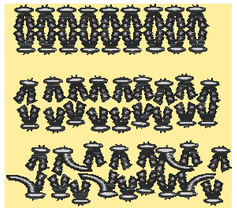
D. vulgaris appears to exhibit the mechanisms found generally in halotolerant gram positive bacteria wherein an overall increase in the branched PLFA, both saturated as well as unsaturated was observed. Increase in the major branched chain acids have been postulated to result in the increase of membrane fluidity with increase in salt concentration.





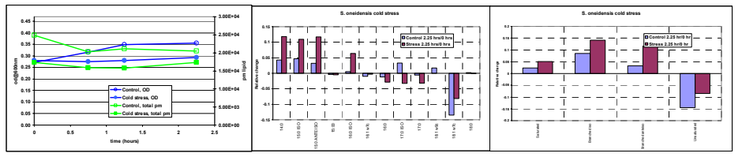


Membrane fluidity can be changed by altering the conformation of the fatty acid tail from the phospholipid molecule. Unsaturated and branched lipids increase the membrane fluidity by decreasing the packing efficiency of the bilayer.



Phospholipid response to Cold Stress

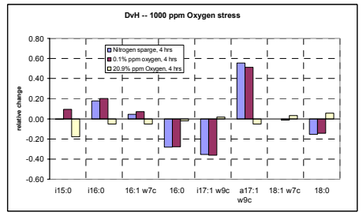
***Shewanella oneidensis* MR1 (SO)**

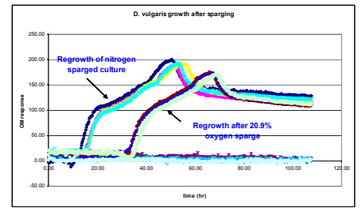


In this SO cold stress experiment, very little growth was observed in either the control or the stress cells. There still were conformational changes observed in the lipids, which were different to those observed for the salt stress. Membranes are less fluid when cold, because the lipid molecules have less kinetic energy. Bacteria may compensate for this by increasing the amount of branched and/or unsaturated fatty acids which prevents the lipids from packing closely together.

Phospholipid response to Oxygen Stress

Desulfovibrio vulgaris





This plot shows the lipid response to oxygen changes. For the 0.1% oxygen sparge experiment, the lipid patterns match those found for the control experiment, implying that the stress had little effect on the lipid composition. For the 20.9% oxygen sparge, there is very little change in the lipid composition over time. It is believed that the cells do not grow or die due to the high oxygen levels, but are coated with polysaccharides and exist in a type of static state until the oxygen levels drop. This is supported by the regrowth observed in the 20.9% oxygen sparged cultures when the stress is removed which show increased lag but similar generation times compared to the control due to the sparging stress.

Conclusions

Phospholipid compositional changes during stress are part of the VIMSS pipeline for understanding the whole cell response to stress. These changes can provide insight and support observed changes in the observed genomic expression and protein production. Also, the analysis has proven useful as an independent measure of biomass as well as a measure of the purity of the culture at the time of sampling.

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Acknowledgement

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through contract DE-AC03-76SF00098 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.